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Feature Review

The role of gasotransmitter hydrogen sulfide in plant cadmium stress responses

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Cadmium (Cd) is a toxic heavy metal that poses a significant risk to both plant growth and human health. To mitigate or lessen Cd toxicity, plants have evolved a wide range of sensing and defense strategies. The gasotransmitter hydrogen sulfide (H_2S) is involved in plant responses to Cd stress and exhibits a crucial role in modulating Cd tolerance through a well-orchestrated interaction with several signaling pathways. Here, we review potential experimental approaches to manipulate H_2S signals, concluding that research on another gasotransmitter, namely nitric oxide (NO), serves as a good model for research on H_2S. Additionally, we discuss potential strategies to leverage H_2S-reguated Cd tolerance to improve plant performance under Cd stress.

Highlights

Hydrogen sulfide (H_2S) is an endogenous gaseous signaling molecule that plays a crucial role in plant growth, development, and responses to environmental stresses.

Plant responses to cadmium (Cd) stress involve various signaling molecules and H_2S has been demonstrated as a crucial regulator.

H_2S can modulate Cd tolerance generally by regulating morphophysiological processes, Cd accumulation, and antioxidant defense.

H_2S functions through complex interaction with various signaling pathways, including nitric oxide, H_2O_2, methane, hormones, and plant metabolites.

Persulfidation of target proteins is a promising direction for elucidating H_2S-modulated Cd tolerance.

H_2S: shift from stench to a gaseous signal under Cd stress

Cd is considered one of the most hazardous environmental pollutants and can cause numerous phytotoxic effects [1–3]. Exposure to Cd can reduce seed germination, seedling growth, plant biomass, and crop yield. It can impair essential physiological processes such as nutrient and water uptake, photosynthesis, chlorophyll synthesis, stomatal conductance, transpiration, and electron transport [1,2,4,5]. These effects typically result from a combination of multiple toxic mechanisms, making it challenging to fully understand Cd phytotoxicity mechanisms. The best documented mechanisms include: (i) excess production of reactive oxygen species (ROS) and induction of oxidative stress, (ii) chromosomal aberrations, (iii) change in expression of genes and DNA damage, and (iv) disturbance of apoptosis and autophagy [5,6].

Plants have evolved a series of mechanisms or strategies to cope with Cd toxicity [4,6,7]. Cd, when accumulated in plants, is detoxified via cytoplasmic chelation and vacuolar sequestration, or via less understood cell wall detoxification mechanisms that enable plants to sequester Cd in the cell wall, where most root Cd resides [4,8]. When Cd enters the cells, plants activate their defense systems, which include diverse antioxidant enzymes and antioxidants, osmolytes, and phytochelatins (PCs) [2,7,9]. Recent studies have shown that plant responses to Cd involve various signaling elements, and the activation of detoxification mechanisms requires the participation of signaling molecules, such as ROS, NO, phytohormones, carbon monoxide (CO), and melatonin [1,6,10]. Furthermore, H_2S, a third gaseous signaling molecule, has been recently documented as a key regulator in plant Cd tolerance [11–13].

H_2S, a colorless gas with a pungent smell of rotten eggs, was first described in 1713 as being poisonous and has long been recognized as hazardous to all forms of life and the environment [14]. It was not until the late 20th century that H_2S was identified to act as an endogenous signal in plants. This discovery led to a paradigm shift from H_2S being regarded as a mere toxin to it being recognized as an important physiological regulator of plant processes [14,15]. Indeed, H_2S has been established to exhibit regulatory effects on a wide range of physiological processes, from seed germination to stress responses and senescence [15–18]. Recent studies have focused on the

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protective functions of H_2S in mitigating plant Cd stress [1,13,19,20]. Cd-induced changes of endogenous H_2S generation have been reported in several plants, suggesting a link to Cd tolerance [1,11,19]. Through application of exogenous donors, H_2S was revealed to facilitate Cd tolerance and mitigate its detrimental effects on various plants via diverse mechanisms [8,20,21]. Moreover, crosstalk between the H_2S signal and other signaling molecules has been often reported under Cd stress [10,22,23]. This has prompted extensive research on plant signaling crosstalk in response to Cd stress. Despite significant progress in recent years, our understanding of the mechanisms remains rudimentary. Here we summarize recent progress in: (i) our understanding of H_2S metabolism and its modulation in plants, (ii) the roles of H_2S in plant Cd stress responses, and (iii) interplay of H_2S with other signaling molecules under Cd stress. Prospects for future research concerning utilization of H_2S in improving Cd tolerance in plants are also discussed.

Endogenous biosynthesis and exogenous application of H_2S in plants

Endogenous H_2S biosynthesis and detection in plants

In plants, endogenous H_2S biosynthesis occurs in different subcellular compartments (Figure 1) [14,24]. H_2S metabolism primarily occurs in the chloroplast, including the cytosol and mitochondria, with compartmented pools that appear to communicate [24,25]. In chloroplasts, H_2S is

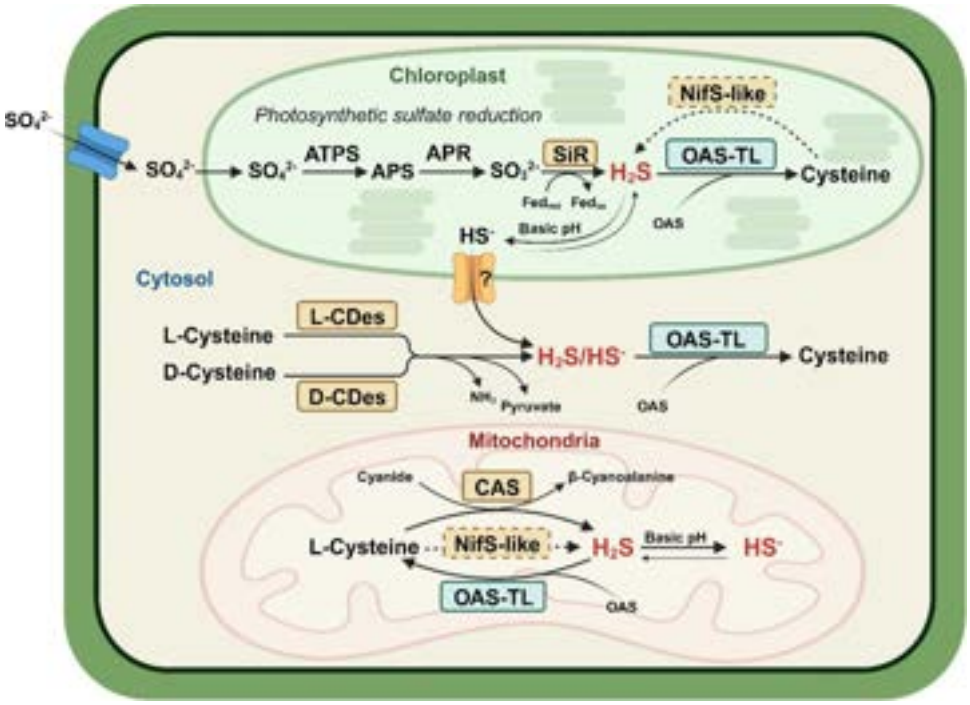

Figure 1. Model for H_2S biosynthesis in different subcellular locations in plant. The major source of H_2S is associated with the photosynthetic sulfate reduction pathway in the chloroplast, where sulfate (SO_4^{2-}) absorbed through sulfate transporters is reduced by ATP sulfurylase (ATPS) to adenosine 5′-phosphosulfate (APS), further by APS reductase (APR) to sulfite (SO_3^{2-}), and then by sulfite reductase (SiR) to produce H_2S [15,25]. H_2S can be further catalyzed by *O*-acetylserine(thiol)lase (OAS-TL) incorporation with *O*-acetylserine (OAS) to synthesize cysteine, while the same reaction also exists in mitochondria and cytosol [24–26]. In cytosol, L/D-cysteine desulfhydrase (L/D-CDes) and L-CDes 1 (DES1), which are members of the OAS-TL family, can produce H_2S from L/D-cysteine [14]. In mitochondria, H_2S is produced during the detoxification of cyanide to β-cyanoalanine by cyanoalanine synthase (CAS) at expense of L-cysteine [29]. Nitrogenase Fe-S cluster (NifS) is a putative enzyme that catalyzes cysteine to produce H_2S in chloroplast and mitochondria [14]. In addition, excess H_2S in chloroplast and mitochondria can be dissociated into its ionic form (HS^-) at basic stroma pH [24]. Figure created with BioRender.com.

generated as an intermediate in photosynthetic sulfate reduction and is a by-product of cysteine synthesis. When absorbed from the environment through sulfate (SO_4^{2-}) transporters, sulfate is continuously reduced in the chloroplast by ATP sulfurylase (ATPS) to form adenosine 5′-phosphosulfate (APS), then by APS reductase (APR) to form sulfite (SO_3^{2-}), and finally to produce H_2S by sulfite reductase (SiR) with ferredoxin as an electron donor [15,25]. The generated H_2S can freely permeate the membrane and diffuse to other cellular compartments or participate in reactions catalyzed by *O*-acetylserine(thiol)lyase (OAS-TL) enzymes condensed with *O*-acetylserine (OAS) to synthesize cysteine [24]. Various other OAS-TLs share responsibility for cysteine synthesis, with the other major isoforms located in the cytosol and mitochondria [25,26]. In addition, excessive H_2S can be dissociated into the charged form of HS^- at a relatively basic stromal pH (8), which cannot permeate the membrane freely and, as such, requires the activity of a currently unknown transporter protein [24]. Moreover, cytosol has the highest cysteine concentration > 300 μM, which is 30 times higher than that of other compartments [27], making it a major site of H_2S produced from cysteine. Different types of cysteine-degrading and H_2S-releasing enzymes are localized in this compartment [14,24]. Cysteine desulfhydrases (CDes), including L-cysteine desulfhydrases (L-CDes) and D-cysteine desulfhydrases (D-CDes), are critical cytosolic enzymes that are associated with the regulation of plant growth and stress responses [14,28]. The L-CDes and D-CDes degrade L- and D-cysteine, respectively, releasing H_2S, ammonia (NH_3), and pyruvate [17,28]. In addition to cytosol, L-CDes are present in mitochondria and chloroplasts, whereas D-CDes are predominantly localized in the cytosol [29]. Furthermore, the nitrogenase Fe-S cluster (NifS), which has L-CDes-like activity, may also generate H_2S in the chloroplasts and mitochondria [14]. The mitochondria-localized β-cyanoalanine synthase (CAS) can generate H_2S by detoxifying cyanide to β-cyanoalanine at the expense of L-cysteine [14,25]. Similar to chloroplast, the generated H_2S can also synthesize cysteine as catalyzed by the mitochondrial isoform of OAS-TL, thus forming a cyclic pathway [24]. Similarly, excess H_2S produced in mitochondria also accumulates in the HS^- form and reaches an equilibrium with H_2S [24,25] (Figure 1). In arabidopsis (*Arabidopsis thaliana*), thiosulfate/3-mercaptopyruvate sulfur transferase 1 (MST1) also generates H_2S [30]. Corpas *et al.* [31] reported the presence of peroxisomal H_2S, however, its source remains unknown. These results suggest the existence of intricate mechanisms that regulate H_2S generation in plant systems. Similar to NO signaling, by orchestrating enzymes and scavenging pathways localized in diverse subcellular compartments, plants may effectively organize H_2S signals in plant cells [32,33]. Adopting the stringing and careful approaches recently employed by the plant NO community to work with gasotransmitters is considerably beneficial [32–34].

To advance our understanding of the signaling role of H_2S in plants, accurately measuring *in vivo* H_2S signals both temporally and spatially is urgently required. Various methods exist for the detection of H_2S in plants, including colorimetric assays [35,36], ion-selective electrodes (ISEs) [37], amperometric electrodes (PHSSs) [38], and fluorescent probes [39,40]. The advantages and limitations have been discussed elsewhere, with the fluorescence imaging approaches, particularly near-infrared (NIR) fluorescence imaging, considered more promising because of their noninvasive and real-time capabilities [25,39,41,42]. Further progress in developing more accurate, specific, reliable, real-time, and user-friendly detection methods is required.

Exogenous H_2S donors and application

Exogenous H_2S donors (H_2S-releasing agents) and inhibitors (including H_2S scavengers) play vital roles in unraveling the roles of H_2S in modulating plant growth and stress response [1,18]. The development of H_2S donors that could mimic the endogenous release profile and potential inhibitors (including scavengers) would be of great importance. At present, various H_2S donors and inhibitors (including scavengers) have been identified or developed [18,43]. Here, only those that have been applied in plants are summarized (Table 1).

Table 1. Exogenous H_2S donors applied in plants

Donors	Applications	Advantages	Drawbacks
Sulfide salts (NaHS, Na_2S)	Widely applied in various plant species [15,43,49]	-Direct and instant release of H_2S -No by-product -Cheap	- Uncontrollable release rate -Short burst of H_2S, different from endogenous enzymatic H_2S production
GYY4137	Widely applied in various plant species [25,43]	-Slow release of H_2S -Commercially available	-Slow hydrolysis rate in water -Difficult to distinguish the effect with by-products
Dialkyldithiophosphates	Maize [45]	-Slow and controlled release of H_2S -Environmentally friendly	-Poor solubility in water -Few studies in plants
Ap39	*Arabidopsis thaliana* L. [46]	-Mitochondrial-targeted -Continuous and controlled release of H_2S	-Mechanism of action is not very distinct -Few studies in plants
NOSH-aspirin/NOSH	*Medicago truncatula* L. [48]; *Medicago sativa* L. [47]	-Release of NO and H_2S -No odor smells -Synergistic effect -Environmentally friendly	-Challenging to distinguish the effect between individual priming molecules -Few studies in plants to date
N-(benzoylthio) benzamide derivatives (5a, 8l, 8o)	*Azolla pinnata* [49]	-Stable release in aqueous buffers -No detectable release to the air of 5a	-Need to be activated by thiols -Few studies in plants

Inorganic sulfide salts, and especially NaHS, are the most widely applied H_2S donor in numerous plants and have greatly promoted research on the physiochemical roles of H_2S [15,17]. Sulfide salts provide a rapid and direct H_2S burst without generation of a complicating by-product [44]. However, they fail in providing a continuous emission of H_2S at a control rate that mimics the endogenous enzymatic generation [44]. When hydrolyzed in aqueous solution, the equilibrium ratio between H_2S, sulfide ions (S^{2-}), and bisulfide ions (HS^-) species depends largely on pH, temperature, and pressure [43]. Moreover, the instantaneous release of H_2S may lead to higher content of such species and cause nocuous effects [44]. Therefore, the instant release property of sulfide salt may lead to inconsistent or even opposite effects and donors with continuous controlled release rate are highly preferred due to their ability to better mimic the physiological situation. Morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate (GYY4137) is such a slow-releasing H_2S donor usually applied in parallel with NaHS and exhibiting similar effects in plants [17,44]. However, the release of H_2S from GYY4137 is temperature- and pH-dependent: less H_2S is released at neutral or basic pH or low temperature (<4°C), while higher amounts are released if pH <3 [44]. However, its slow rate of hydrolysis renders it challenging to distinguish observed effects from H_2S, intact GYY4137, and any by-products following hydrolysis. Indeed, one of the by-products, dichloromethane, can be metabolized to CO, which is another gasotransmitter [43]. Another set of slow controllable H_2S-releasing donors are dialkyldithiophosphates and disulfidethiophosphates [25,45]. The major drawback is poor solubility in water, which might play a potential role when loading into hydrophobic parts of plant cells [43]. With a mitochondrial-targeting motif, 10-oxo-10-(4-(3-thioxo-3H-1,2-dithiol-5-yl)phenoxy) decyl) triphenylphosphonium bromide (AP39), which releases H_2S slowly at a controlled rate, could be a useful tool to explore the specific function of H_2S at the subcellular level [25,46]. That being said, its use is in its infancy in plant systems and requires further exploration. Other novel H_2S donors reported in plants are NOSH-aspirin and NOSH, which could release H_2S and NO. This has thus been proposed as a promising priming agent against stress conditions in plants [47,48]. However, evaluating the physiological role of either signal is complicated by the fact that both are released simultaneously, so its use for investigating mechanistic aspects is challenging. Besides, Yamasaki *et al*. [49] recently reported an application of several thiol-triggered donors (5a, 8l, and 8o) in *Azolla pinnata*, which also merits further study.

Exogenous H_2S inhibitors and scavengers exhibit opposite effect to H_2S donors. The inhibitors mainly block the function of H_2S generation enzymes which may also interfere with other metabolic

processes, while scavengers directly target H_2S [43]. The most commonly used inhibitors in plants include potassium DL-propargylglycine (PAG), pyruvate (PP), hydroxylamine (HA), and aminooxyacetic (AOA) [50–53]. Among various scavengers, hypotaurine (HT) is most widely used in plants [19,53,54]. Overall, significant progress has been made recently in the field of H_2S donors and inhibitors (including scavengers), but a more widespread application of these reagents in plants is clearly merited.

Roles of H_2S in plant responses to Cd stress

Endogenous H_2S levels and biosynthesis in plants under Cd stress

Cd triggers alteration of endogenous H_2S generation in various plants grown under different culture conditions exposed to short- and/or long-term Cd stress (Table 2). Indeed, changes in

Table 2. Cadmium-induced H_2S generation in various plant species

Plant species	Culture conditions	Cd treatment	Exposure duration	Tissues	H_2S level[a]	Responsible enzymes	Refs
Medicago sativa	Hydroponic solution	200 μM $CdCl_2$	12 h	Roots	↑	l-CDes, d-CDes	[50]
	Hydroponic solution	100 μM $CdCl_2$	12 h	Roots	↑	l-CDes	[52]
Cynodon dactylon	MS liquid medium	100, 250, 500, 750, 1000, 2000, 5000 μM $CdCl_2$	12, 24, 48 h	Whole plant	↑	l-CDes	[55]
Arabidopsis thaliana	1/2 MS medium	50 μM Cd^{2+}	2 days	Whole plant	↑	l-CDes, d-CDes	[63]
	1/2 MS medium	100 μM $CdCl_2$	72 h	Roots, leaves	↑	l-CDes	[64]
	1/2 MS medium	25, 50, 100, 150 μM $CdCl_2$	5 days	Roots	↑	l-CDes, DES	[60]
	1/2 MS medium	25, 50, 75 μM $CdCl_2$	10 days	Whole plant	↑	d-CDes	[11]
Oryza sativa	Hydroponic solution	250, 500 μM $CdCl_2$	3 days	Leaves	↑	–	[54]
Brassica rapa	Nutrient soil vermiculite mixture	5,10,20 mM $CdCl_2$	24 h	Roots	↑	d-CDes, DES	[61]
	Petri dishes with solution	4 μM of $CdCl_2$	3, 6, 12, 24, 48, 72 h	Root tips	↑	l-CDes, d-CDes	[57]
	Hydroponic solution	10 μM $CdCl_2$	72 h	Root tips	↑	d-CDes	[110]
Nicotiana tabacum	Germinated in petri dishes	20 μM $CdCl_2$	72 h	Roots	↑	d-CDes	[58]
Brassica napus	Hydroponic solution	20 μM $CdCl_2$	7 days	Roots	↓	l-CDes	[1]
Solanum lycopersicum	Hydroponic solution	5 μM $CdCl_2$	7 days	Roots	↑	l-CDes	[19]
		25 μM $CdCl_2$			↓	l-CDes	
Capsicum annuum	Pots with perlite	0.1 mM $CdCl_2$	5 weeks	Leaves	↑	–	[66]
Vigna radiata	Germinated in petri dishes	200 μM $CdCl_2$	10 days	Leaves	↑	l-CDes, d-CDes	[65]
Cucumis sativus	Germinated in petri dishes	200 μM $CdCl_2$	48 h	Root tips	↑	–	[36]
Brassica juncea	Pots soil	200 mg kg^{-1} $CdCl_2$	30 days	Leaves	↑	l-CDes, OAS-TL	[94]
Triticum aestivum	Pot with sand perlite mixture	0.1 mM $CdCl_2$	4 weeks	Leaves	↑	–	[71]
Allium sativum	Hydroponic solution	10^{-4}, 10^{-3} M $CdCl_2$	7 days	Leaves	↑	–	[62]
		10^{-2} M $CdCl_2$			↓		
Salix matsudana	Pots with soil	150 mg kg^{-1} $CdCl_2$	40 days	Leaves	↑	–	[12]
	Hydroponic solution	5, 10, 30 μM $CdCl_2$	60 days	Roots and leaves	↑	–	[56]

[a]↑ in the table means increase, ↓ in the table means decrease, – in the table means not mentioned.

endogenous H_2S content were suggested to be associated mostly with Cd tolerance in plants [11,12,50]. In some plant species, Cd was reported to induce continuous H_2S generation and facilitate changes in intracellular H_2S level, with exogenous donors being demonstrated to alleviate Cd toxicity effects and increase Cd tolerance [50,55,56]. However, in *Brassica rapa*, the continuous H_2S increase was responsible for root inhibition, one of the hallmark symptoms of Cd toxicity, at concentrations as low as 4 μM [57]. Similarly, a toxic effect of H_2S was also reported in *Nicotiana tabacum* when exposed to 20 μM Cd [58]. This may be related to the developmental stage of plants combined with concentrations of H_2S released, as H_2S can be toxic at high cellular doses [16,59]. In some studies, different concentrations of Cd consistently induced significant increases of H_2S content at the root, leaf, and whole plant levels [11,54,55,60,61]. However, dose-dependent discrepancies in results were reported in *Solanum lycopersicum* roots [19] and *Allium sativum* leaves [62], with a lower dose of Cd causing increases, while higher doses cause decreases, in the H_2S content. A decrease in H_2S content was also observed in rapeseed seedling roots [1]. When taken together, these observations indicate that endogenous H_2S levels in Cd-stressed plants can be ascribed to various factors, including: (i) Cd concentrations; (ii) plant species, tissues, or organs; (iii) the developmental stage at which stress is perceived; and (iv) duration of stress exposure. It has additionally been speculated that H_2S, as a signaling molecule, may be elicited rapidly by Cd and the signal relayed to other tissues/organs due to its high lipophilic nature, or alternatively metabolized through methylation in the cytosol or rapid oxidation in mitochondria [1,24]. Therefore, understanding both the kinetics and subcellular location of H_2S generation may be of great help in understanding its oscillations and role in plants under Cd stress. More accurate and real-time detection methods will also greatly aid. By contrast to the differential H_2S production profile in these studies, they all demonstrated a protective role of H_2S in Cd-stressed plants via application of optimal concentrations of H_2S donors, scavengers, or biosynthetic inhibitors.

The sources for Cd-triggered H_2S seem to have similar origin of either L-CDes or/and D-CDes (Table 2); one point is that the induction of the enzymes needs different regulators. Liu *et al*. [63] and Zhang *et al*. [11] reported the activation of *LCD* and/or *DCD* expression and increase of H_2S production by Cd-induced transcript factor WRKY in arabidopsis. Qiao *et al*. [64] suggested that salicylic acid (SA) could activate L-CDes activity and thus enhanced H_2S production in arabidopsis exposed to Cd stress. In Bermuda grass, the release of H_2S in response to Cd stress was reported to be dependent on NO [55]. An enhancement of H_2S biosynthesis enzymatic activities was also observed on application of calcium (Ca) [65] and methane (CH_4) [52], while the opposite effect was observed following the application of cinnamaldehyde (CA) [58]. Although enzymatic activities were not determined, an increase in H_2S content was observed in *Capsicum annuum* following exogenous silicon application, which suggests a possible regulatory role of silicon in H_2S biosynthesis [66]. As the role of H_2S during plant stress responses, including Cd stress, is closely associated with cellular H_2S, more in-depth studies on the metabolism and homeostasis of H_2S during stresses are required. These studies also suggested crosstalk within or between signaling molecules and other regulators during the plant Cd response and we will discuss this in more detail later.

Exogenous H_2S regulates morphophysiological responses of plants under Cd stress

Despite the controversial function of endogenous H_2S in the regulation of plant responses to Cd stress, a large number of studies have now shown that optimal concentrations of exogenous H_2S can alleviate the detrimental effects of Cd stress on plants (Table 3). NaHS is the most commonly used exogenous H_2S in modulating plant Cd stress (Table 3). Exogenous NaHS either pretreated or treated simultaneously with Cd, fumigation, spraying, or seed priming can improve plant growth attributes such as: (i) seed germination, (ii) root and shoot biomass or length, (iii) leaf

Table 3. Exogenous H_2S application method and its effect on plants' responses under Cd stress[a]

Plant species	Cd stress and duration	H_2S donor and concentration	Application methods	Morphological responses	Physiological responses	Possible mechanisms	Refs
Medicago sativa	100 μM; 12 h or up to 72 h	NaHS; 100 μM	Pretreatment for 6 h	Mitigated growth inhibition	Alleviated lipid peroxidation and loss of membrane integrity; reduced root Cd accumulation	Increased activities and transcripts of SOD, POD while decreased that of APX; interacted with NO signal	[81]
Populus euphratica	100 μM; 72 h	NaHS; 50 μM	Pretreatment of cells for 6 h	Not mentioned	Mitigated Cd-stimulated PCD; reduced cytoplasmic Cd while increased vacuolar Cd; alleviated lipid peroxidation; reduced H_2O_2 accumulation	Upregulated antioxidant enzyme activity; decreased Cd influx through the H_2O_2-activated PM calcium channels; stimulated vacuolar Cd sequestration via activating tonoplast Cd^{2+}/H^+ antiporters	[76]
Triticum aestivum	2mM; 48 h	NaHS; 0.9 mM	Pretreatment for 12 h	Alleviated germination inhibition	Reduced disturbance of PM integrity, superoxide radicals, H_2O_2, and MDA contents	Increasing activities of amylase, esterase, POD, CAT, APX and inhibited that of lipoxygenase; promoted free amino acid accumulation	[67]
	100 μM; 4 weeks	NaHS; 200 μM	Treated simultaneously for 4 weeks	Alleviated growth inhibition	Increased leaf chlorophyll content and Fv:Fm ratio; decreased oxidative stress and proline content; decreased Cd uptake and translocation	Enhanced activities of SOD, CAT, POD and uptake of some essential mineral nutrients; interaction with NO signaling	[71]
	50 μM; 5 days	NaHS; 50 μM	Pretreatment for 5 days	Mitigated growth inhibition	Protected photosynthetic apparatus; increased soluble sugar content; alleviated ROS accumulation and oxidative damage; reduced Cd content	Increased photosynthetic and PSII electron transport rate; upregulation of gene expression related to photosynthetic carbon assimilation and sucrose synthesis, downregulation of those related to sucrose decomposition	[20]
Brassica napus	100, 500 μM; 15 days	NaHS; 100, 200 μM	Treated together with Cd	Improved plant growth and root morphology	Improved chlorophyll content and photosynthetic activity; alleviated MDA and ROS accumulation; improved cell structures with well-developed organelles	Improved ion and element uptake; enhanced SOD, POD APX, CAT, and GR activity in leaves and roots	[13]
	20 μM; 1 week	NaHS; 50 μM	Pretreatment for 6 h	Improved plant growth and leaf chlorosis	Improved chlorophyll content; increased root Cd retention and decreased leaf Cd accumulation	Increased root pectin content and demethylation	[1]
Brassica rapa	50 $mg \cdot kg^{-1}$	NaHS; 1.5 mM	Seed priming for 12 h	Alleviated seed germination and growth inhibition	Decreased ROS, EL, MDA, MG levels; enhanced LRWC and photosynthesis; improved pigment contents;	Increased activities of SOD, POD, CAT, APX; increased proline content; regulated minerals and Cd homeostasis	[73]

(continued on next page)

Table 3. (continued)

Plant species	Cd stress and duration	H_2S donor and concentration	Application methods	Morphological responses	Physiological responses	Possible mechanisms	Refs
Brassica chinensis	6.24 mg kg^{-1}; 50 days	NaHS; 5 ml of 0.3 mM	Sprayed every 3 days since produced two leaves	Mitigated growth inhibition	Alleviated ROS accumulation, oxidative stress, and photosynthesis inhibition	Improved antioxidant enzyme activity; increased GSH content and ratio of GSH:GSSG, AsA:DHA; improved soil catalase activity	[78]
Oryza sativa	250, 500 μM; 3 days	NaHS; 100 μM	Treated together with Cd	Alleviated growth inhibition, leaf chlorosis, and rolling	Alleviated the reduction of ROS and MG accumulation, oxidative stress, photosynthetic pigments, and soluble protein; maintained leaf water status	Decreased Cd accumulation; elevated AsA and GSH contents and redox status; inhibited proline accumulation; upregulates antioxidant enzymes; enhanced glyoxalase enzyme activity	[54]
Foxtail millet	5 mM; 24 h	NaHS; 50 μM	Fumigated for 24 h	Alleviated Cd-induced toxic symptoms of leaves	Reduced EL, MDA, H_2O_2 contents; enhanced photosynthesis	Increased proline content and P5CR activity and gene expression, decreased PDH activity and gene expression	[51]
Cucumis sativus	100 μM; 24 h	NaHS; 100 μM	Pretreated for 24 h	Not mentioned	Improved photosynthetic parameters	Stimulated ATP hydrolysis and proton transport, and alleviated V-ATPase inhibition; interaction with H_2O_2 signaling	[100]
	200 μM; 48 h	NaHS; 100 μM	Pretreatment for 24 h	Alleviated growth inhibition	Reduced cell death, oxidative damage, and ROS accumulation in root tips;	Enhanced antioxidant enzyme activity; inhibited mitochondrial Cty c release and the opening of the MPTP	[68]
	200 μM; 48 h	NaHS; 100 μM	Pretreatment for 24 h	Alleviated growth inhibition	Alleviated cell death; maintained mitochondrial function	Reduced mitochondrial H_2O_2 accumulation; increased ATPase activity and downregulated *CsVDAC* and *CsANT* expression	[36]
Arabidopsis thaliana	40 μM;	NaHS; 0.3 mM	Sprayed daily for 8 days	Alleviated leaf chlorosis and growth inhibition	Alleviated Cd-induced oxidative damage and ROS accumulation	Decreased the soluble Cd fractions in plants; facilitated the sequestration of protoplast Cd into the cell wall	[75]
Salix matsudana	5, 10, 30 μM; 60 days	NaHS; 0.3 mM	Presprayed on root and left for 10 min	Alleviated biomass reduction	Reduced MDA and H_2O_2 accumulation	Reduced the soluble Cd fractions; reduced Cd distribution in organelles and vacuoles and increased that in the cell wall; increased antioxidant enzyme activity and gene expression; increased GSH content and endogenous H_2S	[56]

Table 3. (continued)

Plant species	Cd stress and duration	H_2S donor and concentration	Application methods	Morphological responses	Physiological responses	Possible mechanisms	Refs
Hordeum vulgare	5, 25 μM; 25 days	NaHS; 200 μM	Treated together for 25 days	Alleviated growth inhibition	Increased leaf chlorophyll and soluble protein contents; reduced MDA content	Elevated SOD activity in leaf and root; decreased CAT and APX in leaf; reduced Cd concentration	[70]
	5, 25, 50 μM; 24 h	NaHS; 200 μM	Pretreated for 2 days	Not mentioned	Reduced H_2O_2 and $O_2^{\bullet-}$	Increased GSH and AsA contents	[70]
Isatis indigotica	4.5, 13.5, 22.5 μM; 14 days	NaHS; 50, 100, 150, 200 μM	Treated together for 14 days	Alleviated inhibition on root and shoot length	Decreased Cd transport from root to shoot and promoted Cd accumulation in root; decreased Cd influx and proportion in organelles	Improved cell wall component synthesis and Cd fixation; inhibited Cd transmembrane movement; altered Cd chemical forms; increased metallothioneins	[8]
Nicotiana tabacum	50 mg l^{-1}; 15 d	NaHS; 0.3, 0.6, 0.9, 1.2 μM	Sprayed onto the blade surfaces once every 3 days up to 15 days	Not mentioned	Repaired Cd-induced photosynthetic damage, ROS generation, and cell membrane damage	Increased gene expression and activity of antioxidant enzymes; increased gene expression and secretion of plant complexins and resistant proteins to promote chelate formation; promoted absorption of K^+ and Ca^{2+}, forming antagonisms with Cd^{2+} and reducing Cd uptake; interacted with CaM signal	[22]
Zingiber officinale Roscoe	7.5 mg l^{-1}; up to 10 days	NaHS; 0.8 mM	Spraying every 24 h	Not mentioned	Decreased ROS; increased pigment contents during the early stage; inhibited the decrease in the Pn, Gs, Ls, and increase in Ci	Increased antioxidant enzymes activities (APX, GR, MDAHR, and DHAR); upregulating *ZoNramp1* and *ZoPCS1* genes in roots; downregulated *ZoHMA2* gene in rhizomes and roots	[77]
Miscanthus sacchariflorus	50 μM; 3 days	NaHS; 10, 25, 50, 100, 300, 400, 500 μM	Pretreatment for 1 day	H_2S < 300 μM: mitigated growth inhibition; H_2S > 400 μM: aggravated Cd toxicity	H_2S < 300 μM: reduced ROS accumulation and oxidative damage; improved photosynthetic indicators; H_2S > 400 μM: aggravated ROS accumulation and oxidative damage	H_2S < 300 μM: enhanced the AsA-GSH cycle by regulating the activities of antioxidant enzymes; reduced Cd-elevated osmoregulatory substances; H_2S > 400 μM: enhanced the TF by reducing GST activity and decreasing Cd chelating ability	[74]
Leucaena leucocephala	5 ppm; 3 or 7 days for radical length measurement	NaHS; 1, 50, 100 μM	Treated together for 3 or 7 days	Increased radical length	Reduced ROS accumulation, oxidative damage, and chlorophyll content; improved K and Cu uptake	Increased Cd content in root and decreased Cd content in shoots as well as TF	[69]

(continued on next page)

Table 3. (continued)

Plant species	Cd stress and duration	H_2S donor and concentration	Application methods	Morphological responses	Physiological responses	Possible mechanisms	Refs
Trigonella foenum-graecum	1.0, 1.5, 2.0 mM; 2 weeks	NaHS; 100, 150, 200 μM	Seed priming	Improved seedling growth	Improved LRWC, Ci, Pn, Gs, and stomatal transpiration; reduced H_2O_2 production and EL	Enhanced antioxidant enzyme activity; regulated phenolic and flavonoid content; induced Put and Spd biosynthesis	[80]
S. matsudana Koidz	150 mg kg^{-1}; 40 days	NaHS; 50 mg kg^{-1}	Mixed with Cd in soil for 40 days	Mitigated growth inhibition	Alleviated ROS accumulation and oxidative damage; reduced Cd concentration	Enhanced antioxidant enzyme activity and relative expression of stress-related genes; restored the redox status of AsA and GSH and improved glyoxalase II activity	[12]
Matthiola incana	50 mg kg^{-1}	NaHS; 1.5 μM	Root irrigation at 4-day intervals	Mitigated growth inhibition	Improved photosynthetic parameters and leaf gas exchange traits; alleviated oxidative damage and H_2O_2 accumulation	Reduced Cd accumulation; increased soluble sugar content and antioxidant enzyme activity	[114]

[a]Abbreviations: CaM, calmodulin; Ci, intercellular CO_2 concentration; EL, electrolyte leakage; Fv/Fm, maximum quantum efficiency of photosystem II; Gs, stomatal conductance; LRWC, leaf relative water content; MG, methylglyoxal; MPTP, mitochondrial permeability transition pore; PC, phytochelatins; PCD, programmed cell death; P5CR, proline-5-carboxylate reductase; PDH, proline dehydrogenase; Pn, net photosynthetic rate; Put, putrescine; SOC, soil organic carbon; Spd, spermidine; TN, total nitrogen; V-ATPase, vacuolar H^+-transporting ATPase.

chlorosis, and (iv) root architecture under Cd stress conditions. The possible physiological basis underlying this may be that application of H_2S can: (i) reduce ROS accumulation, (ii) alleviate Cd-induced oxidative damage, (iii) improve nutrient uptake, (iv) affect Cd accumulation, (v) enhance photosynthetic ability, (vi) modify osmotic balance, and (vii) protect cell structure (Table 3). For example, exogenous H_2S application reduced Cd-induced oxidative damage and increased wheat seed germination [67], cucumber root elongation [68], plant height, and leaf area of *Salix matsudana* Koidz [12] through direct scavenging of ROS by the antioxidant defense system [8]. Improved physiological function of the mitochondria and thus suppressed cell death have been reported in the root tips of *Cucumis sativus* [36]. Exogenous NaHS can improve nutrient uptake, for example, it increased K^+, Ca^{2+}, and Mg^{2+} uptake, and the Ca^{2+}:Mg^{2+} ratio, while it reduced the Na^+:K^+ ratio in *B. napus* [13], and improved K and Cu homeostasis in *Leucaena leucocephala* [69], thus promoting plant growth. Furthermore, exogenous NaHS can alter Cd accumulation and translocation in the various tissues of plants [1,8,12,22,70,71] and will be discussed in detail later.

Enhanced photosynthetic ability is an important characteristic of exogenous NaHS-mediated function in Cd-stressed plants [13,54,71–73]. Ali *et al*. [13] reported improved cell structure with well-developed chloroplasts following treatment with exogenous NaHS in *B. napus*. Exogenous H_2S in various plant species improved pigment content, that is, chlorophyll a, chlorophyll b, and carotenoids, as well as various photosynthetic parameters, leaf relative water content (LRWC), and transpiration rate (Table 3). Furthermore, exogenous NaHS can protect the photosynthetic apparatus from Cd stress by increasing heat dissipation and maintaining leaf osmotic balance by regulating genes related to carbon assimilation and sugar metabolism in wheat [20]. In *L. leucocephala*, 100 μM NaHS treatment reduced chlorophyll content and did not affect carotenoid content [69]. However, this may be because exogenous NaHS increased Cd uptake

in roots, which likely has deleterious effects on plant biomass and pro-plastid synthesis [69]. Additionally, adding NaHS to Cd-stressed plants altered soluble protein, soluble sugar, and proline contents [20,54,70,71,73,74]. These responses may aid in maintaining the cellular osmotic pressure balance, thus enhancing plant tolerance to Cd toxicity. These findings suggest that the protective effects of H_2S may involve regulating multiple morphophysiological responses.

Mechanisms involved in H_2S-mediated Cd stress response

Involvement of Cd accumulation

H_2S alleviates Cd toxicity in plants by regulating Cd accumulation, which may involve several mechanisms modulating Cd uptake, translocation, subcellular distribution, and chemical forms (Figure 2) [22,75,76]. For example, exogenous NaHS upregulated the expression of *ZoNramp1* in roots and *ZoPCS1* in both roots and rhizomes, whereas it downregulated that of *ZoHMA2* in roots and rhizomes [77]. Spraying of NaHS reduced Cd uptake in tobacco by promoting mineral nutrient (K^+ and Ca^{2+}) absorption and antagonizing with Cd^{2+} [22]. This H_2S supplementation could also increase the expression of PC genes, including *Pr2* and *Pr8*, and promote plant complexin and resistant protein secretion to stimulate chelate formation and vacuole sequestration [22]. In *Populus euphratica* cells, exogenous NaHS regulated cellular Cd homeostasis by enhancing Cd sequestration into the vacuole by activating tonoplast Cd^{2+}/H^+ antiporters and

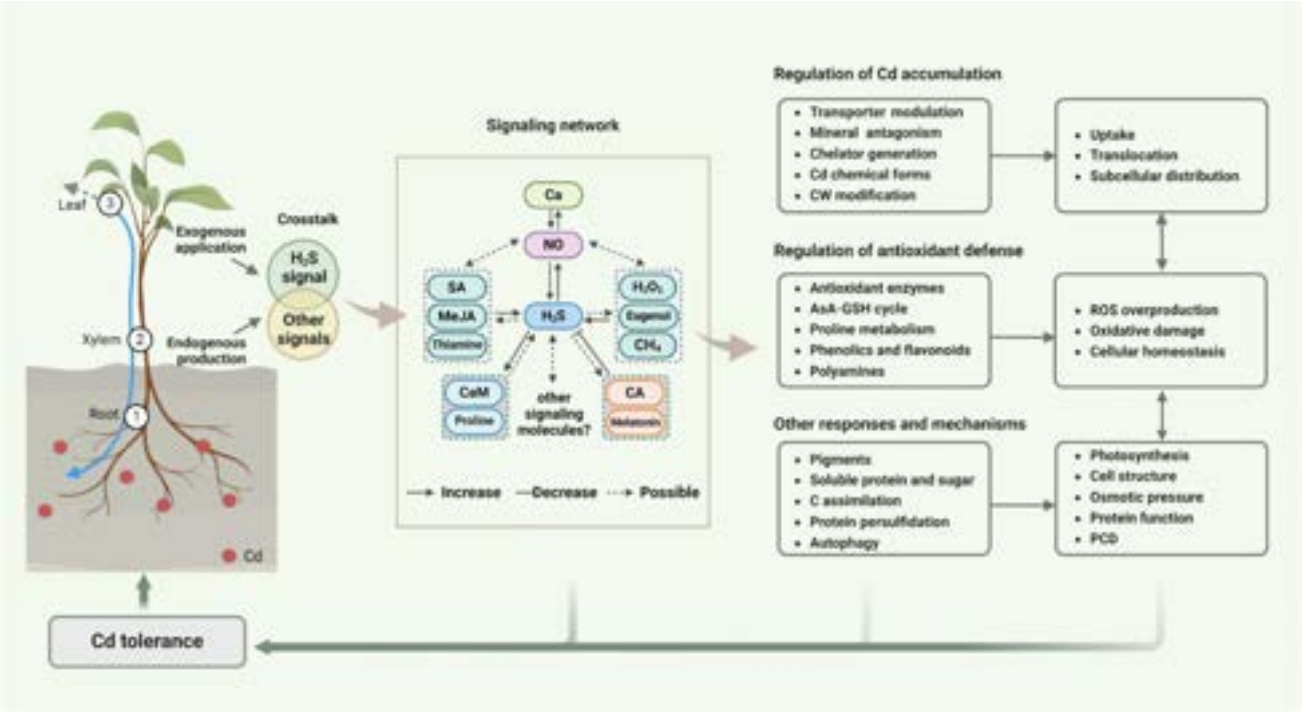

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Figure 2. Functions of H_2S in regulating plant Cd stress response. The H_2S signal can be endogenously induced or exogenously intensified. It interacts with many other signaling components, forming a complex signaling network where they synergistically or antagonistically work together to enhance Cd tolerance. H_2S regulates several mechanisms involved in Cd uptake, translocation, and subcellular distribution, such as transporter expression, mineral uptake, chelator generation, Cd chemical forms, as well as CW modification to reduce Cd accumulation. H_2S also enhances antioxidant defense through antioxidant enzymes, AsA-GSH cycle, and other antioxidant metabolites to inhibit ROS overproduction and maintain cellular homeostasis. It regulates photosynthesis parameters, C assimilation, and osmotic regulators in response to Cd stress. In addition, H_2S may function by modulating protein persulfidation and autophagy to enhance Cd tolerance in plants. Abbreviations: AsA-GSH, ascorbate-glutathione; C, carbon; CA, cinnamaldehyde; Ca, calcium; CaM, calmodulin; Cd, cadmium; CH_4, methane; CW, cell wall; H_2S, hydrogen sulfide; MeJA, methyl jasmonate; NO, nitric oxide; PCD, programmed cell death; ROS, reactive oxygen species; SA, salicylic acid. Figure created with BioRender.com.

decreasing Cd influx through H_2O_2-activated plasma membrane (PM) Ca channels [76]. Furthermore, NaHS spraying modified Cd subcellular distribution in arabidopsis by altering Cd chemical forms [75]. Exogenous NaHS could significantly decrease soluble Cd fractions while increasing the insoluble fractions, thus promoting Cd sequestration from sensitive protoplasts into the cell wall [75]. Altered chemical forms of Cd and decreased Cd proportions in the organelles have also been reported in *S. matsudana* [56] and *Isatis indigotica* [8]. Moreover, H_2S could restrict the long-distance translocation of Cd from roots to shoots, thus decreasing Cd accumulation in the upper parts of plants [1,8]. In *B. napus*, H_2S increased root Cd retention by elevating pectin content and pectin methylesterase activity, thus increasing Cd binding sites in the root cell wall [1]. In *I. indigotica,* the restricted translocation was due to increased generation of Cd chelators PC and metallothionein and altered content of cell wall components in the roots [8]. Additionally, coapplication of the rhizosphere bacteria *Azospirillum brasilense* and exogenous NaHS reduced the transportation factor (TF) and Cd content by decreasing the available soil Cd content and enhancing root fixation [78]. Despite multiple strategies reported for H_2S-regulated Cd accumulation, the underlying mechanisms still remain largely unknown. Further research is required to understand their roles in the modulation of metal transporters.

Involvement of antioxidant capacities

H_2S also alleviates Cd toxicity in plant by reducing ROS overproduction, oxidative damage, and maintaining cellular homeostasis [3,21,72,73,79]. These beneficial effects are implemented by mobilizing plants' complex antioxidant defense systems, which mainly include enzymatic and nonenzymatic antioxidants [20,28,51,70,73,80]. For example, exogenous NaHS significantly upregulated the total activity of antioxidant enzymes, including ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and glutathione reductase (GR), reducing lipid peroxidation and H_2O_2 accumulation [76]. This could further suppress Cd influx through PM Ca channels activated by H_2O_2, thus alleviating Cd toxicity [76]. Similarly, enhanced superoxide dismutase (SOD), CAT, POD, and APX activities have been reported in *B. napus* [13], *B. rapa* [73], and *C. sativus* [68]. Furthermore, the gene expression and activities of the antioxidant enzymes SOD, POD, and CAT were notably upregulated by NaHS in tobacco [22]. In alfalfa seedling roots, exogenous NaHS differentially modulated the total activities and corresponding transcripts of antioxidant enzymes, including increases in SOD and POD and decreases in APX, thus alleviating Cd-induced oxidative damage [81]. Differentially regulated antioxidant enzymatic activities have also been reported in *Hordeum vulgare*, in which exogenous NaHS enhanced SOD activity in leaves and roots, whereas it decreased CAT and APX in leaves [70]. Although numerous studies have highlighted the role of H_2S in orchestrating antioxidant enzymatic activity under Cd stress, the molecular mechanisms underlying its action are not yet fully understood. One of the proposed mechanisms may occur through H_2S-mediated persulfidation, which involves converting the protein's thiol group (–SH) into the persulfide group (–SSH). Similar to protein nitrosylation induced by NO, this post-translational modification (PTM) of proteins may lead to alterations in enzymatic structures, activities, and cellular localizations [82,83]. Some persulfidation proteins have also been identified and demonstrated to be involved in various processes, including modulating gene expression, autophagy, phytohormone signaling, and interaction with other PTMs [84–87]. However, unlike protein nitrosylation in NO-regulated Cd tolerance [88,89], the target proteins and sites of persulfidation underlie the H_2S-regulated Cd tolerance lag.

The regulation of the ascorbate-glutathione (AsA-GSH) cycle also contributes to the protective effects of NaHS against Cd-induced ROS accumulation and oxidative stress. The application of NaHS notably increased the reduced GSH and AsA contents in Cd-stressed barley [70] and pak choi [78]. Furthermore, the AsA-GSH cycle involves multistep enzymatic reactions in which four enzymes, namely APX, GR, monodehydroascorbate reductase (MDHAR), and dehydroascorbate

(DHAR), coordinate to modulate metabolism [77]. Elevated activities of these four enzymes following NaHS supplementation were demonstrated in ginger during the early treatment stages [77]. In Cd-stressed rice, H_2S intensified the activity of DHAR and GR, while maintaining the activity of APX and MDHAR above the control level in the presence of enhanced SOD and CAT, thereby amending the level and redox status of AsA and GSH and contributing to the control of ROS generation and oxidative damage [54]. Similarly, H_2S elevated APX, MDHAR, and DHAR activity, thereby restoring the AsA-GSH redox status and facilitating Cd detoxification in *S. matsudana* [12].

H_2S could also modulate the levels of small molecule antioxidants to protect against Cd phytotoxicity. NaHS application induced proline accumulation, which can stabilize protein structures and cell membranes, and maintain cellular homeostasis, thus alleviating the oxidative damage caused by Cd [51,73]. The H_2S-increased proline content was probably due to NaHS increased activity and transcript levels of proline-5-carboxylate reductase (P5CR), while it decreased those of proline dehydrogenase (PDH) [51]. In addition, H_2S improved seedling growth under Cd stress by increasing phenolic and flavonoid content and stimulating the biosynthesis of putrescine and spermidine [80]. Taken together, these results indicate that H_2S could maintain cellular redox homeostasis by facilitating the antioxidant defense system, thereby alleviating Cd-induced toxicity and enhancing plant tolerance to Cd stress.

In addition to the aforementioned mechanisms, other potential mechanisms associated with H_2S-regulated Cd tolerance may exist. For example, H_2S is essential in regulating autophagy through protein persulfidation, which might function in preventing programmed cell death (PCD) during plant adaptation to Cd stress [7,90]. Further research using state-of-the-art multi-omics approaches is needed, as these will likely facilitate the elucidation of the molecular mechanisms underlying H_2S-regulated Cd tolerance in plants.

Interaction of H_2S with other signaling components

Interactions between signaling molecules and plant growth regulators, such as plant metabolites, are crucial in many developmental processes in plants and survival under stress conditions [7,33,53,79,91,92]. Prominent progress has been made in understanding the interaction between H_2S and NO, H_2O_2, CH_4, Ca signaling [Ca^{2+}, calmodulin (CaM)], hormones [SA, methyl jasmonate (MeJA), melatonin], and plant metabolites (thiamine, CA, eugenol, and proline), which act synergistically or antagonistically to enhance Cd tolerance in plants (Figure 2).

Interaction between H_2S and NO

H_2S and NO are two intimate collaborators that regulate plant growth, development, and abiotic stress tolerance [33,93]. Studies have mainly focused on stress physiology and their interaction cascades in response to Cd exposure. Exogenous H_2S and/or NO donors can improve endogenous levels and alleviate toxicity symptoms under Cd stress in various plant species, whereas the opposite occurs when inhibitors and/or scavengers are applied [55,71,94]. These alleviative effects can be achieved by enhancing antioxidative capabilities [55,71], PCs and non-protein thiol production [94], ATP synthase activity [95], Cd accumulation [71,81], and the uptake or use of some essential nutrients [71,94]. In the roots of *Medicago sativa* and leaves of *Triticum aestivum* seedlings, the effect of NaHS was reversed by 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO, a specific NO scavenger), suggesting that NO is a downstream component of H_2S in Cd stress response [71,81]. However, for Cd-stressed *Cynodon dactylon* leaves, the NO signal was blocked by cPTIO as well as H_2S scavenger and/or inhibitors; however, the ameliorative effects of NaHS were not reversed by cPTIO addition, indicating that NO acts upstream of H_2S to enhance Cd tolerance [55]. Similar results have been reported in the leaves of Cd-stressed *Brassica juncea* [94] and *S. lycopersicum* [95], where cPTIO, HT, and PAG reversed the positive effects of exogenous

NO. These findings imply that H_2S and NO are indispensable for eliciting defense mechanisms to enhance Cd tolerance. The interplay between them appears rather complex, as H_2S can act upstream or downstream of NO in the signaling cascade, but little is known about the mechanisms by which they affect each other. Moreover, H_2S and NO can function through PTMs like persulfidation and *S*-nitrosation that compete with each other and with other PTMs (*S*-sulfenylation, *S*-cyanylation, *S*-acylation, *S*-glutathionylation, Tyr-nitration, and carbonylation) [33,83,96,97]. However, little is known about the molecular mechanisms that modulate Cd tolerance. Future in-depth investigations are required to comprehensively understand this crosstalk and its roles in regulatory processes.

Interaction between H_2S and H_2O_2

The function of H_2S has generally been linked to that of H_2O_2 in the regulation of stress tolerance because of the central role of H_2O_2 in the response of plants to adverse environmental conditions [98,99]. H_2S may act upstream or downstream of H_2O_2 signaling cascades, synergistically or antagonistically, during stress responses as well as in normal physiological processes [91,98,99]. Under Cd stress, H_2S was mostly reported to reduce H_2O_2 production and alleviate Cd-induced oxidative damage in plants [13,20,70,76]. Several mechanisms underlying this process have been proposed [70,73,76]. However, to the best of our knowledge, little is known about the synergistic action of H_2S and H_2O_2 signaling in the Cd stress response. However, Kabała *et al*. [100] reported that exogenous H_2O_2 could increase H_2S signaling independent of desulfhydrase and protect V-ATPase from Cd-stressed cucumber roots. However, in Cd treatment alone, the induced H_2O_2 exerted opposite effects on H_2S by regulating V-ATPase [100]. This may be due to the H_2O_2 concentration, as H_2O_2 itself can show either signaling or toxic effects in a dose-dependent manner.

Interaction between H_2S and CH_4

H_2S has also been reported as a vital downstream signal of CH_4, which is also proposed as a new candidate gasotransmitter in regulating several physiological processes and Cd stress responses [52,101]. Kou *et al.* [101] were the first to report the crucial role of L-CDes-dependent H_2S in CH_4-elicited adventitious root development in cucumbers. Subsequently, Mei *et al*. [102] demonstrated that H_2S operates downstream of CH_4 to trigger lateral root formation by modulating cell cycle regulatory gene expression. A similar interplay cascade was also found in Cd-stressed alfalfa, in which the attenuated Cd toxicity symptoms disappeared after endogenous H_2S removal by HT or PAG [52]. CH_4 could stimulate L-CDes activity to intensify endogenous H_2S production and enhance Cd tolerance through limiting Cd influx and accumulation and enhancing antioxidant defense [52]. Future research is needed to clarify their interactions and the underlying mechanisms in Cd stress and other physiological processes and stress conditions.

Interaction between H_2S and Ca signaling

H_2S-mediated Cd stress alleviation is also accompanied by Ca signaling, among which Ca^{2+} and CaM are the core components [22,103]. In plants, they modulate metabolism and signal transduction under various stress conditions [103,104]. In Cd-stressed *Vigna radiata* seedlings, Khan *et al.* [65] revealed that exogenous Ca profoundly reduced Cd content and activated the biosynthesis of H_2S and NO. Further inclusion of scavengers and chelators indicated that H_2S acted downstream of the Ca and NO signals, whereas Ca acted both upstream and downstream of NO. Thus, H_2S, NO, and Ca cooperated to enhance antioxidant enzymatic activity, PC content, and AsA-GSH cycle components to reduce Cd-induced impairments [65]. In tobacco, exogenous H_2S improved the uptake of Ca^{2+} and the expression of CaM to synergistically initiate a series of tolerance mechanisms, including increasing the expression of PC genes and antioxidant enzymes, as well as uptake of minerals, to improve Cd detoxification [22]. In addition, H_2S acts downstream of Ca-dependent protein kinase (CDPK) signal to improve Cd tolerance in

arabidopsis [105]. CDPKs are crucial protein kinases that amplify the Ca^{2+} signal in plants through protein phosphorylation [103,105]. Similar to L-CDes-knockout mutants, TFP (CDPK inhibitor) treatment or CDPK mutation reduced L-CDes enzyme activity and H_2S content. Moreover, exogenous H_2S pretreatment upregulated the expression of Cd stress-responsive genes (*CAX3*, *MYB107*, *PCS1*, *POX1*, and *MT3*) in *cdpk3* mutants, proving the interaction between H_2S and CDPKs in the plant Cd stress response [105].

Interaction between H_2S and hormones

The interactions between H_2S and phytohormones modulates various physiological processes and stress responses [53,92]. Recently, the involvement of their interplay in mediating Cd stress alleviation has been reported [92,106,107]. H_2S likely acts downstream of several hormones under Cd stress [64,106–108]. In arabidopsis, pretreatment with SA increased L-CDes, intensified H_2S production, and enhanced Cd tolerance. These positive effects disappeared in *lcd* mutant plants, suggesting that H_2S acts downstream of SA [64]. In another study, the combined treatment with H_2S and SA exhibited more profound effects in facilitating antioxidant defense and nutrient balance to confer Cd tolerance than when applied independently [106]. Induction of H_2S signal was also reported following exogenous MeJA treatment in Cd-stressed foxtail millet [108]. However, the regulation of endogenous H_2S induction remains unclear. Gu *et al*. [109] proposed a possible interplay between H_2S and melatonin in mediating Cd tolerance responses. To the best of our knowledge, direct evidence for this has recently been reported in wheat, where melatonin exerts antagonistic effects with H_2S and synergistically with NO to protect plants tolerant to Cd poisoning. This interaction may function by stimulating nitrate reductase and nitrite reductase and inhibiting L-CDes and D-CDes activities, respectively [107]. Furthermore, although no direct evidence has been shown, it is speculated that H_2S may fine-tune the ethylene signal [7] to adapt to Cd stress.

Interaction between H_2S and plant metabolites

Plant metabolites interact with H_2S to mitigate Cd stress. In foxtail millet, exogenous H_2S further elevated the proline level, which can act as a heavy metal chelate, ROS quencher, and osmoprotectant during Cd detoxification. These elevated levels are achieved by increasing the transcript levels and activities of P5CR and PDH. Moreover, the combined application of H_2S and proline further improved tolerance compared with a single treatment, suggesting that H_2S acted upstream of and cooperated with proline in protecting against Cd stress [51]. In *B. rapa*, a eugenol to H_2S to GSH signaling cascade has been suggested to confer Cd tolerance [110]. Eugenol is a phenolic aromatic compound with various bioactivities, including antioxidative, anti-inflammatory, antimicrobial, anticancer, and antiviral activities [111]. Eugenol treatment could trigger H_2S emission by upregulating the activities of L-CDes and D-CDes and the genes *BrLCD* and *BrDCD* that encode them. However, all positive effects were compromised by H_2S scavengers or biosynthesis inhibitors. These results indicate that H_2S is a necessary downstream component of eugenol signaling for intensifying the GSH pool to reduce Cd accumulation, oxidative damage, and growth inhibition [110]. Another important bioactive compound, CA, interplays with H_2S in relieving Cd stress in tobacco roots but by reducing endogenous H_2S generation [58]. Thiamine, an enzymatic cofactor in universal metabolic pathways and an activator of defense responses, has recently been demonstrated to interact with H_2S during Cd stress in strawberry [112,113]. Foliar spraying of thiamine could further increase H_2S and NO in leaves as a route to improve antioxidant capacity and mineral homeostasis, as well as inhibit Cd accumulation, whereas this effect was partially compromised by HT. These results suggest that H_2S is an essential downstream component of thiamine signaling and a possible interactor of NO [113].

In summary, these studies shed new light on the interaction between H_2S and many other signaling components; however, as yet this information is far from complete. The role of their crosstalk,

underlying molecular basis, and the interactions with other signaling components need to be elucidated in the future.

Concluding remarks and future perspectives

H_2S, a well-known gasotransmitter in plants, plays an essential role throughout the plant life cycle as well as in their response to adverse conditions. Tremendous breakthroughs have been made in recent years toward deciphering H_2S metabolism and functions in plant response to Cd stress. Cd can trigger H_2S signal from multiple pathways and L/D-CDes were suggested as the main enzymes responsible for H_2S generation in most plant species. Most of the studies indicated that H_2S could facilitate plant tolerance to Cd stress, although some studies have reported toxic effects from increased endogenous H_2S levels, which might relate to the level and rate of diffusion. The protective effect of H_2S on regulating plant performance exposed to Cd toxicity has been widely documented by exogenous administration of optimal concentrations of exogenous H_2S donor, NaHS. The well-known mechanisms identified so far include regulation of Cd accumulation behaviors and antioxidant capacities by mediating various processes. Unraveling how H_2S modulates Cd translocation in differential plant tissues is of particular interest for developing crop varieties with low Cd accumulation in edible parts and improving phytoremediation capabilities. Furthermore, H_2S was also shown to interact with many other signaling components in regulating plant tolerance to Cd toxicity, but the complex network of regulatory mechanisms is far from fully understood.

To achieve a comprehensive mechanistic understanding of how H_2S mediates plant responses to Cd and to accelerate its practical application in agriculture, future research should focus on the following aspects (also see Outstanding questions).

i. Metabolism and detection of endogenous H_2S. Although some possible metabolic pathways have been suggested for H_2S synthesis in plants under Cd stress, how Cd triggers H_2S metabolism and the specific molecular mechanisms are still unclear. Moreover, dual functions of endogenous H_2S have been reported in plant responses to Cd stress, but whether they are differentially induced remains unknown. Another significant challenge is the development of effective methods for real-time monitoring of the dynamic spatial and temporal changes, as well as subcellular localization, of H_2S *in vivo*. In addition, two or more methods should be used simultaneously to detect H_2S levels at the same time to ensure accuracy, as different methods may result in magnitude difference in determined biological H_2S levels.
ii. H_2S targets and signaling network. As a signaling molecule, H_2S is proposed to regulate Cd tolerance through stimulating target regulators (genes, proteins, and metabolites) that are not yet fully understood. The development of more advanced multi-omics approaches and integrated analysis could help identify those targets and associated metabolic pathways, offering insights for genetic manipulation to improve plant Cd tolerance. A promising direction and study hotspot is persulfidation, as H_2S is reported to directly modify protein cysteine thiol groups through *S*-sulfhydration. Further exploration and characterization of persulfidated proteins, possible 'persulfidase', as well as the underlying selective mechanisms will significantly advance our understanding of H_2S function in plant Cd response. Furthermore, H_2S interacts with many other signaling substances, which are also involved in regulating plant Cd response. Understanding the interaction between these factors will facilitate a comprehensive insight of the mechanisms underlying H_2S functions in plants under Cd stress.
iii. Novel H_2S donors and multidimensional study. Although various H_2S donors have been developed and applied in plants, NaHS remains the most widely used donor in Cd stress studies. However, NaHS cannot mimic the spatio-temporal fluctuation of endogenous H_2S generation in plants. Thus, there is a need to study and develop novel donors with more desirable

Outstanding questions

How does Cd trigger the H_2S signal and what are the underlying molecular mechanisms?

What are the mechanisms that determine the differential role of endogenously produced H_2S?

How can the real-time spatio-temporal changes of H_2S generation in plants be effectively measured and the effects be mimicked by using exogenous donors?

What are the target regulators (genes, proteins, and metabolites) of H_2S in regulating Cd tolerance?

How do persulfidated proteins contribute to the amelioration of Cd stress in plants? Is there a putative 'persulfidase' involved in the process?

What is the molecular basis for the crosstalk networks between H_2S and other signaling components?

characteristics. Additionally, current investigations on the impact of exogenous H_2S on Cd stress in plants have typically focused on specific stress stages or tissues, such as root and shoot during short-term responses. Future research should explore multiple tissues and organelles, especially edible parts of crop, at various growth stages and throughout the plant's life cycle.

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Declaration of interests

The authors declare no competing interests.

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